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The timing of exogenous ethylene supplementation differentially affects stored sweetpotato roots

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Abstract

The effects of continuous supplemental ethylene (10 μL L^{-1}) timing on the physiology and biochemistry of sweetpotato roots during storage at 25 ^{o}C were examined. Alongside continuous ethylene or air treatments, a subset of the roots were transferred at dormancy break, from those previously stored in ethylene into air and vice-versa. The study showed distinctive ethylene-induced effects on the metabolism of individual sugars, phenolic compounds and phytohormones (abscisic acid and zeatin riboside) across the spatial gradient of the root flesh and skin tissues. Although ethylene fumigation doubled root respiration, sprout growth was significantly suppressed. Supplementation of roots with ethylene after dormancy break effectively inhibited sprout growth as much as continuous ethylene alone. On the other hand, truncating ethylene application after dormancy release promoted vigorous sprout growth. After prolonged storage, ethylene treatment was associated with increased weight loss and incidence of proximal rots. Supplemental ethylene also accelerated the catabolism of monosaccharides, and promoted accumulation of phenolic compounds in the proximal root sections.

Keywords: Respiration, sprouting, sugars, phenolics, abscisic acid

1 Introduction

Despite the economic value and growing contribution of sweetpotato (*Ipomoea batatas* (L.) Lam.) to improved nutrition and health in tropical and sub-tropical countries, it presents a challenge as a staple food due to its relatively short postharvest life. No viable alternatives to cold storage are yet available for the long-term storage of sweetpotato in the tropics. This poses a major challenge in its food security role and marketing across the globe (Hall et al., 1998; Cheema et al., 2013).

The phytohormone ethylene has been ascribed important roles in the regulation of many physiological and biochemical mechanisms in plant tissues that affect the quality of horticultural produce. Consequently, many techniques to improve the storage of a variety of crops are based on elucidating the mediating role of ethylene. Current understanding suggests that ethylene may elicit variable (sometimes contrasting) responses in plant tissues depending on a matrix of factors, including the type of tissue, growth stage, the ethylene concentration and timing of exposure. The effects of ethylene on climacteric fresh produce are well established. Presently, there are also on-going studies on the effects of exogenous ethylene in mediating dormancy, sprout growth and senescence mechanisms in low ethylene producing commodities like potatoes and onion, which have demonstrated that continuous ethylene supplementation could be a practical alternative to suppress sprouting (Prange et al., 1998; Daniels-Lake et al., 2005; Downes et al., 2010; Cools et al., 2011; Foukaraki et al., 2014; Foukaraki et al., 2016a). Based on these findings, and given its convenience as a natural plant hormone,

there has been interest in the use of ethylene as an option for extending the storage life of sweetpotato (Cheema et al., 2013). However, the optimal timing of ethylene supplementation and its detailed effects on the physiological and biochemical profiles in sweetpotato remains uncertain.

In the study reported herein, sweetpotato roots were treated with continuous ethylene (10 μL L^{-1}) at different timings before or after endodormancy break. The effects of the treatment options on physiological quality attributes as well as the spatial profiles of non-structural carbohydrates, individual phenolic compounds and endogenous phytohormones in the roots were assessed during storage.

2 Materials and methods

2.1 Plant material

Three separate consignments of North Carolina-derived sweetpotato cv. Covington grown in different commercial farms in the USA were supplied by Barfoots of Botley Ltd. (W. Sussex, UK). The first consignment for Experiment 1 was a previously stored crop (ca. 8 months after harvest on delivery), planted in July 2011 at Jim Summelin Farm (North Carolina, USA) and harvested four months later in October 2011. The roots were cured (30 ^{o}C, 90 % relative humidity, 7 days) and shipped to Barfoots of Botley Ltd. where they were stored at 14 ^{o}C as a commercial stock. Samples were taken from the commercial storage and delivered to Cranfield University in July 2012. Root samples for the second (2a) and third (2b) experiments were from the same harvested stock (in October 2012) from Anderson East Farm (North Carolina, USA) but supplied at different times after curing as described before. The supply for Experiment 2a was

delivered 10 days after harvest to Cranfield University via Barfoots while that for Experiment 2b was initially held in storage at Barfoots (14 ^{o}C) before delivery to Cranfield University in February 2013. Thus there was a planned 5 months cold storage interval between Experiments 2a and 2b.

2.2 Experimental design

Root samples for Experiment 1 were stored in either continuous ethylene (10 μL L^{-1}) or continuous air alone while those for Experiments 2a and 2b were divided and stored under four concerted regimes of ethylene treatment viz. (1) continuous ethylene (10 μL L^{-1}) supplementation throughout storage; (2) truncated ethylene (10 μL L^{-1}) supplementation after dormancy break, followed by storage in air; (3) post-dormant ethylene (10 μL L^{-1}) supplementation after previous air storage and (4) continuous air storage, respectively (Foukaraki et al., 2016a). Each treatment was carried out in triplicate in water-sealed, 81.2 L polypropylene boxes. The boxes were covered with transparent lids and flushed with the aforementioned concentrations of air/ethylene mix, respectively. Certified ethylene gas inertized with nitrogen (BOC, Surrey, UK) of stock concentration 5000 μL L^{-1} and pressurized into a cylinder (50 L water capacity, 20 MPa) was diluted to 10 μL L^{-1} with air from a compressor (Hydrovane) using mass flow controllers operated at ambient temperature under a pressure of approximately 241 kPa (MFC SierraTek and control unit Sable Systems International, USA). Pure air and the ethylene+air mix (10 μL L^{-1}) were applied to the crop storage boxes via gas distribution manifolds (HNL Engineering Ltd., UK) and flexible nylon tubing. The gases were bubbled through bowls of water placed in the boxes to maintain a relative

humidity of 70 % to 95 %. Tropical-like climatic conditions were maintained by keeping the room temperature constant at 25 ^{o}C. The humidity and temperature in the boxes were monitored with Gemini data loggers (Tiny-tag Ultra 2, 0-95 % relative humidity, -25 ^{o}C to 85 ^{o}C temperature, Part No. TGU-4500). Exhaust gas was disposed to the outside of the building through a ventilated extraction hole in the ceiling of the store room. The exhaust tubes were connected to the side of the boxes opposite to the inflow gas to provide a continuous, diagonal flow-through at flow rates that maintained the CO_2 concentration below 0.5 % (Reid and Pratt, 1972). CO_2 levels in the boxes were regularly checked as described by Cools et al. (2014) by injecting headspace gas samples into a gas chromatograph (GC model 8340, DP800 integrator, Carlos Erba Instruments, Herts, UK) fitted with a Hot Wire Detector (HWD), analytical Porapak column (2 m length, 6 mm O.D., 4 mm I.D.) and calibrated with 10 % CO_2, 2 % O_2 balanced with nitrogen (BOC, Surrey, UK).

Periodically, ethylene concentration in the headspace gas in each box was analysed as previously described by Terry et al. (2007). A 60 mL plastic syringe was used to withdraw and inject the gas samples (ca. 10 mL per injection) into a gas chromatograph fitted with flame ionisation detector (FID, 250 ^{o}C) (analytical Porapak column of 2 m length, 6 mm O.D., 4 mm I.D.) to ascertain a concentration of ca. 10 μL L^{-1} in the ethylene-flushed boxes and ca. 0 μL L^{-1} in the air-flushed boxes. The calibration standard was 10.3 μL L^{-1} ethylene balanced with air (BOC, Surrey, UK). Accordingly, the mass flow controllers were adjusted until the said concentrations in the respective boxes were achieved.

In Experiment 1, the effect of the treatments on root respiration, weight, sprout growth and decay were the only parameters assessed while in Experiments 2a and 2b, the effect

on biochemical profile (sugars and phenolics) were additionally evaluated. Furthermore, selected roots in Experiment 2a were analysed for phytohormones. To facilitate measurements, each box in Experiments 2a and 2b contained two subsamples of sweetpotato roots; non-destructive and destructive. The 'non-destructive' subsamples were numbered individually for physiological quality assessment while the 'destructive' subsamples were randomly selected for destructive spatial analysis of sugars, phenolic compounds and phytohormones at each outturn. Dormancy break was defined as the time point when approximately 10 % of roots in the control treatment had sprouts ≥ 1.0 mm.

2.3 Non-destructive assessments

The non-destructively tested roots [Experiment 1 (n=45), Experiment 2a (n=21) and Experiment 2b (n=30)] were numbered individually and repeatedly assessed for weight, decay and sprout growth. The measured weight at each sampling event was expressed as a percentage of the initial root mass. The number of decayed roots was also expressed as a percentage of the initial number placed in each box. Decayed roots were removed from the stock after counting but taken into account in determining cumulative decay in the subsequent outturns (Rees et al., 2003). The number of sprouted roots, the mean number of sprouted buds per root and the maximum sprout length (mm) per root were also recorded. A sprout was defined as any bud growth ≥ 1.0 mm.

2.4 Biochemical assays

Destructive biochemical assays were preceded with determination of the respiration of selected roots using a Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, Las Vegas, USA) as described by Collings et al. (2013) with slight modifications. Individual roots were randomly picked from the treatment boxes and immediately weighed and incubated in 3 L, air-tight glass jars, connected in series to a carbon dioxide detector (CA-10, Firmware version 1.05), an oxygen detector (FC-10, Firmware version 3.0) and a water vapour pressure detector (RH-300) of the Sable System equipment to measure CO_2 (%), O_2 (%), and water vapour pressure respectively, in the effluent gas. The jars were continuously flushed with air in the push mode with an 80 HP pump (HIBLOW, Techno Takatsuki Co. Ltd., Japan). The dynamic flow-through mode was used to avoid any stress-induced changes as the same roots were used for subsequent biochemical assays. An auto-sampling program was developed with a flow multiplexer to sample the effluent gas from the jars in sequence for 5 min each, allowing 2 min equilibrating time between sampling from the jars. Each jar was sampled a total of three times, giving three readings of CO_2, O_2 and water vapour pressure. Simultaneously, flow rate and barometric pressure were recorded which were used in software calculations. The respiration rates (measured in terms of CO_2 evolution) were analysed with ExpeData Release 1.3.8, Version: PRO software which utilized the flow rate, water vapour pressure and barometric pressure readings for corrections to equivalent CO_2 in dry air at ambient conditions. Subsequently, respiration rate (g kg^{-1} s^{-1}) was calculated taking into account the root weight and time of incubation. Immediately after measuring respiration, the roots were processed to measure the effects of the treatments on non-structural carbohydrates and phenolic

compounds in the spatial segments viz. the proximal (stem end) and distal (root end) of both skin and flesh tissues.

2.4.1 Sample preparation

After determining respiration, the roots were quickly washed under running tap water and dried with a paper towel. Each root was then cut into the proximal and distal segments (approximately a third of the root length measured from the ends, respectively). Each segment was peeled with a sharp knife to obtain two types of tissue: skin (ca. 1.0 mm to 1.5 mm from the outer surface) and flesh. The skin and flesh tissues were snap-frozen in liquid nitrogen, each divided into two and stored at both -40 ^{o}C and -80 ^{o}C, until further analysis. The frozen tissue (-40 ^{o}C) was weighed (fresh weight = ca. 10 gram per sample) and freeze-dried in the dark for 7 days in a Scan Vac Coolsafe freeze-dryer (Scan Vac, Västerås, Sweden). After lyophilisation, the samples were re-weighed to obtain the dry weight (DW) and the amount of dry matter as a proportion of the fresh weight subsequently calculated. The samples were then ground to powder and returned back to -40 ^{o}C in readiness for non-structural carbohydrates and phenolics assays.

2.4.2 Extraction and quantification of non-structural carbohydrates

Sugars were extracted according to the protocol described by Chope et al. (2007) with slight modifications. Briefly, 150 mg of the powdered samples were weighed into 7 mL polystyrene Bijou vials (Sterilin, Staffs, UK) and 3 mL of HPLC grade extraction solvent added: a volume fraction of methanol (62.5 %) and water (37.5 %). The mixture

was incubated in a shaking water bath (Clifton, UK) at 55 ^{o}C for 15 min. During incubation, the vials were vortexed for 20 s every 5 min (Vortex Genie 2, Scientific Industries, NY, USA) to prevent layering. The extracts were left to cool at room temperature and the supernatant filtered through 0.2 µm pore size syringe driven filters (PTFE, Cronus, Jaytee Biosciences, Kent, UK) and stored at -20 ^{o}C pending further analysis. Before analysis, the samples were diluted (dilution factor 10) with HPLC grade water.

Identification and quantification of sugars were done using Agilent 1260 Infinity HPLC system coupled to Agilent 1260 Infinity Evaporative Light-Scattering Detector (ELSD) (Agilent Technologies Inc., Germany). The stationary phase column was a Prevail Carbohydrate ES 5µ, GRACE, USA (250 mm by 4.6 mm; Part No. 35101; Serial No. N908718) fitted with a security guard cartridge. The mobile phase comprised of HPLC grade water (solvent A) and 100 % acetonitrile (solvent B) mixed according to the gradient program A: 20 % to 50 % for 15 min and 50 % to 20 % for 5 min to facilitate a linear increase/decrease of the amount of water in acetonitrile. A 5 min post run equilibration of the column was allowed at 20 % of A. The HPLC operating conditions were controlled with Chemsoft software (Agilent) to generate a pump flow rate of 1.0 mL min^{-1}, 30 ^{o}C column temperature and 20 µL injection volumes of the calibration standards and the samples. Authentic calibration standards of fructose, glucose, sucrose and maltose were obtained from Sigma-Aldrich Co., (UK). In preliminary trials, sucrose was found to be disparately more abundant in sweetpotato than the other sugars and therefore, separate calibration curves were generated with sucrose standard concentrations ranging from 1.0 g L^{-1} to 2.5 g L^{-1}, while fructose, glucose and maltose were prepared to concentrations 0.5 g L^{-1} to 1.25 g L^{-1}. The abundance of the

identified sugars were calculated by comparison with the standard peak areas using GenStat (VSN International Ltd., UK) calibration models generated for the standard curves.

2.4.3 Extraction and quantitation of phenolic compounds

Phenolic compounds were extracted from 100 mg of the freeze-dried powder using 1.5 mL of acidified aqueous methanol (volume fraction of methanol (70 %) water (29.5 %) HCl (0.5 %)). The mixture was incubated in a shaking water bath at 35 ^{o}C for 1.5 h, being vortexed for 10s every 15 min. The cooled samples were filtered as described for sugars and stored at -20 ^{o}C until further analysis.

The extracts were analysed using an Agilent 1200 HPLC system (Agilent Technologies, Berks., UK) coupled with an Agilent 1200 G1315B/G1365B photodiode array detector. The mobile phase solvents comprised HPLC grade water stabilized with 0.5 % formic acid (line A) and 100 % acetonitrile (line B). A gradient program developed with Chemsoft software was used to induce a linear decrease/increase of the amount of water in acetonitrile (A: 95 % to 72 % for 13 min; 72 % to 30 % for 5 min; and 30 % to 95 % for 2 min) with 5 min post run column equilibration. The stationary phase column was an Agilent Zorbax eclipse XDB-C18 column (4.6 mm by 150 mm, 5µm particle size) fitted with a C18 Opti guard column. The operating conditions were controlled to set the pump flow at 1 mL min^{-1}, autosampler injection volume of 5 µL and column temperature at 25 ^{o}C. Identification of the phenolic compounds was based on their HPLC retention times and the UV-VIS spectral signatures in comparison with those of the pure standards of chlorogenic, caffeic, p-coumaric, ferulic acids (Sigma-Aldrich

Co., UK) and iso-chlorogenic acids A, B and C (Oskar Tropisch, Germany) dissolved in acidified aqueous methanol (as above) to concentrations of 20 mg L^{-1} to 50 mg L^{-1}. The concentrations of the samples were quantified on the basis of the UV absorption as calibrated linearly against the standards.

2.5 Extraction and quantification of hormones

Phytohormones in 150 mg of the freeze-dried powder were cold extracted (-20 ^{o}C, 12 h) with acidified aqueous methanol (as above) and quantified with Ultra-Performance Liquid Chromatography coupled to Quadruple Time of Flight Mass Spectrometry (6540 UPLC/QToF/MS, Agilent Technologies) method as described by Cools et al. (2014) and Ordaz-Ortiz et al. (2015). The extraction mixture (4950 µL) was spiked with 50 µL of deuterated internal standards (d_3-DHZR, d_3-DPA, d_5-ABA-GE, d_3-PA, d_4-ABA) at 400 µg L^{-1}. Solids were separated by centrifugation (Heraeus Labfuge 400R Centrifuge, Thermo Scientific, Fischer, UK) and purified with cartridges (Agilent) to remove lipids and plant pigments. The residue was re-extracted with 2 mL of the extraction solvent for 30 min at -20 ^{o}C, both extractions pooled and purified using ion exchange solid phase extraction (Sep-Pack vac tC18). The cartridges were centrifuged (5560 x g, 15 min, 4 ^{o}C) and the supernatant filtered into 15 mL tubes using 0.2 µm pore size syringe filters (Cronus, Jaytee Biosciences Ltd., Kent, UK). The filtered samples were firstly concentrated for 1 h (22 ^{o}C) in a vacuum evaporator (miVac, Quattro Concentrator, Genevac Ltd., UK) to evaporate the methanol and then freeze-dried (Scan Vac, Västerås freeze-dryer, Sweden) overnight. The dried powder was reconstituted with 1 mL formic acid (1.0 mol L^{-1}), vortexed and loaded into Oasis MCX cartridges (150 mg sorbent, 60

µm particle size) (Waters Corporation, Massachusetts, U.S.A.) previously pre-conditioned with 5 mL of methanol and equilibrated with 5 mL of formic acid solution (1.0 mol L^{-1}). The formic acid was discarded and the retained phytohormones were eluted with methanol (2 mL), followed by 2 mL of 0.35 mol L^{-1} NH_4OH in aqueous methanol (volume fraction of water (40 %) and methanol (60 %)). The eluted solution was purged with nitrogen at room temperature to evaporate organic phase and the residue was freeze-dried overnight (in the dark at -110 ^{o}C). The final dried material was reconstituted with 400 µL of formic acid (0.1 %) in ultrapure water containing 20 ng of d_6-ABA added to evaluate the recovery rate. They were then centrifuged for 5 min at 16060 x g. The supernatant was filtered through 0.2 µm syringe filters into amber silanised vials (Agilent) and stored at -40 ^{o}C pending analysis. Aliquots (5 µL) of the samples were injected into UPLC/QToF/MS system comprising an UPLC, a thermostated column compartment (TCC) operated at 30 ^{o}C, a binary pump system (Agilent Technologies, Berks – UK) and a 6540 UHD accurate mass Quadruple Time of Flight with electrospray ionization source (ESI) to quantify the phytohormones.

Endogenous phytohormones in the root tissues of Experiment 2a previously treated with either continuous ethylene (10 µL L^{-1}) (n=84) or continuous air (n=84) alone, were assayed at different timings before and after dormancy break. Also, only the proximal tissues (flesh and skin) where subsequent sprouting was found to be most abundant were examined. A preliminary assay identified ABA and ZR as the major endogenous hormones in the majority of the roots and therefore, the study was limited to the effect of the exogenous ethylene treatment on these two hormones.

2.6 Statistical analysis

All data were first subjected to Shapiro-Wilk normality test and plotted for residuals to verify the assumptions for the Analysis of Variance (ANOVA). ANOVA tables were generated using GenStat for Windows, Version 14 (VSN International Ltd., Herts., UK) and used to identify statistically significant trends. The means between treatments were separated with the Least Significant Difference (L.S.D.) and declared to be significant at 5 % significance level (P= 0.05).

3 Results and discussion

3.1 Respiration rate

The respiration rate of sweetpotato roots continuously supplemented with ethylene significantly increased by ca. 1.5 fold to 2.0 fold compared to continuous air storage. Ethylene supplementation also elicited a characteristic yet transient respiratory peaking (Figure 1). In the case of the previously stored roots (Experiments 1 and 2b), the ethylene-induced respiratory peak occurred long after dormancy break. In contrast to the ethylene treated roots, respiration of roots in air storage was fairly stable at mean values of

87.8 x 10^{-7} g kg^{-1} s^{-1} and 107.9 x 10^{-7} g kg^{-1} s^{-1} in Experiments 1 and 2b, respectively. On the other hand, respiration of the air-stored fresh roots in Experiment 2a dropped from

93.4 x 10^{-7} g kg^{-1} s^{-1} to a basal level of ca. 35.6 x 10^{-7} g kg^{-1} s^{-1}. When ethylene supplementation was truncated after dormancy break and subsamples of the roots were immediately transferred into air and vice-versa, there was a reciprocal effect as they

swapped their respiration status. The reciprocity of root respiration levels when transferred between ethylene and air provides further compelling evidence that ethylene induces higher tissue respiration, as reported for other similar low ethylene producing storage crops (Huelin and Barker, 1939; Reid and Pratt, 1972; Kitinoja, 1987; Cools et al., 2011; Foukaraki et al., 2014; Cheema et al., 2013).

The biochemical basis for ethylene-stimulated respiration and associated effects has not been definitively explained. However, sweetpotato respiration has been reported to also increase in response to elevated temperature, attack by fungi, wounding and other biotic and abiotic stresses (Saltveit, 1999; Hyodo et al., 2003). Changes in the normal atmospheric composition could impose a stress that can trigger higher respiratory response. Also ethylene treatment caused mild tissue injury in the current study which manifested as proximal tissue splitting in some roots. Ethylene induced tissue wounding in sweetpotato was equally observed by other authors (Stahmann et al., 1966; Pratt and Goeschl, 1969 and Kitinoja, 1987), and in the case of the present study, the wounds healed a few days later. Tissue injury, being a mechanical stress, signalled an increased respiratory response. Generally, elevated respiration in crops during storage has undesirable ramifications as it accelerates metabolic activities and leads to premature senescence.

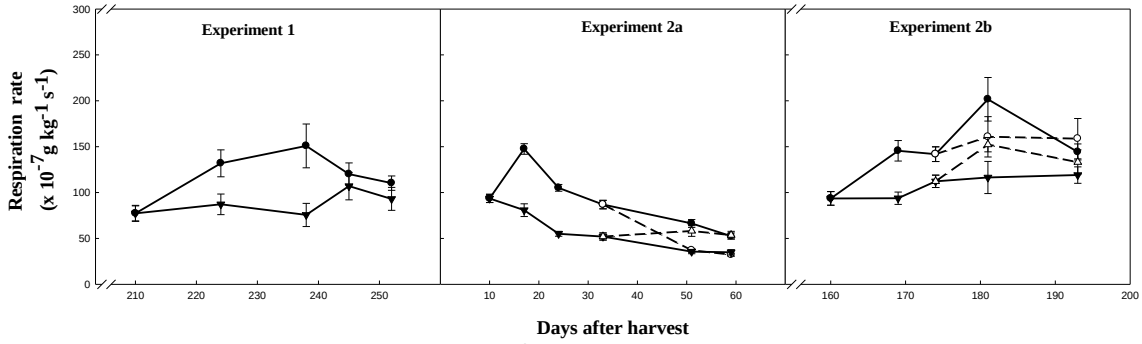

Figure 1. Respiration rate (CO_2 evolution at 25 ^oC) of North Carolina-derived sweetpotato cv. Covington

in (●) continuous ethylene (10 μL L^{-1}); (○) ethylene followed by air; (▼) continuous air; (Δ) air followed by ethylene. Roots for Experiments 1 and 2b were initially held in cold storage (14 ^{o}C) for 210 days and 160 days respectively, after harvest. Experiment 2a and 2b were the same initial consignment. Error bars are shown.

3.2 Hormonal changes and sprout growth

Continuous ethylene supplementation, compared to air storage, significantly reduced sprout growth measured in terms of the percentage of roots that sprouted, number of sprouted eyes per root and the maximum sprout length (Table 1a). As reported for potato (Foukaraki et al., 2014; Foukaraki et al., 2016b), ethylene supplementation either before or after dormancy break achieved an equivalent effect in inhibiting sprout elongation although in the case of sweetpotato, a significantly larger number of sprouts emerged when the roots were treated with ethylene after dormancy break (Table 1b). Truncating ethylene supplementation after dormancy break, and transferring the roots into air exacerbated sprout growth, triggering a large number of long sprouts. Hence once predisposed to ethylene, sweetpotato roots may have to be continually exposed to ethylene or risk compromising quality. This has repercussions on the marketing of sweetpotato previously stored in ethylene as once removed from storage the roots will sprout if not consumed quickly. Differences between continuous air storage and post-dormant transfer from ethylene into air were not significant except for sprout elongation in Experiment 2a for which the latter treatment triggered significantly higher sprout growth (Table 1b).

Table 1a. Effect of continuous ethylene (10 μL L^{-1}) supplementation on sprout growth in North Carolina-derived sweetpotato cv. Covington. Roots were initially stored for 210 days at 14 ^{o}C

before being subjected to the ethylene treatments and stored at 25 ^{o}C. Values shown for each variable are the statistical means for the entire storage duration.

	Experiment 1		
Treatment	% sprouted roots	Max sprout length (mm)	Sprouts per root
Air	31.43	45.19	1.013
Ethylene	6.03	0.38	0.114
l.s.d	8.268	3.67	0.6931
P-value	0.001	< 0.001	0.023

Table 1b. Effect of the timing of ethylene (10 µL L^{-1}) supplementation on sprout growth in North Carolina-derived sweetpotato cv. Covington. Roots for Experiment 2b were initially stored for 160 days at 14 ^{o}C before being subjected to the ethylene treatments and stored at 25 ^{o}C. Swap treatments were effected after dormancy break (i.e. 10 % sprout growth in the air treatments). Values shown for each variable are the statistical means for the entire storage duration.

	Experiment 2a			Experiment 2b		
Treatment	% sprouted roots	Max sprout length (mm)	Sprouts per root	% sprouted roots	Max sprout length (mm)	Sprouts per root
Air	36.7	5.31	1.231	36.7	18.25	1.89
Air to ethylene	32	2.14	0.925	25	4.17	0.838
Ethylene to air	36.4	14.12	1.737	26.7	18.52	1.603
Ethylene	15	2	0.295	5.6	1.31	0.144
l.s.d	17.31	2.793	0.4504	9.06	3.699	0.515
P-value	0.06	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Inhibition of sprout growth with sustained exposure to ethylene and sprouts proliferation following truncated ethylene treatment have been similarly reported in potato and onion (Rylski et al., 1974; Kader, 1985; Prange et al., 1998; Bufler 2009;

Downes et al., 2010; Foukaraki et al., 2014; Foukaraki et al., 2016b). To date, however, there is no conclusive explanation in the literature about the mechanism by which exogenous ethylene retards sprouts when present, and why sprout growth becomes more vigorous when ethylene is removed. More intriguing is the contradiction that ethylene induces increased respiration without the respiratory energy being translated into sprout growth. Cheema (2010) observed a similar discrepancy in sweetpotato cv. Bushbuck and proposed that independent phenomena are involved between exogenous ethylene effect on sprouting and respiration. Respiration rate is generally acknowledged as indicative of the rate of biochemical activities within a crop, as it releases energy for other metabolic processes. That said, the induction by exogenous ethylene of higher respiration while inhibiting sprout growth raises queries about the conceptual view of the tissues being in a quiescent state during dormancy. It appears that many biochemical activities are operational during ethylene-induced dormancy when there is a physiological transition from the endodormant to the ecodormant state.

It was particularly noted in Experiment 2a that, notwithstanding sprout growth inhibition by exogenous ethylene, there was increased biosynthesis of the growth promoting hormone zeatin riboside (ZR) (Figure 2). ZR is a cytokinin associated with cell division and differentiation (Mok and Mok, 2001; Hartmann et al., 2002). The major cytokinin in sweetpotato is ZR and it appears to be a candidate growth signal induced by exogenous ethylene. Notwithstanding the differences between potato and sweetpotato, the effect of exogenous ethylene in boosting ZR content without sprout elongation strengthens the proposition that ethylene also promotes the biosynthesis of other compounds which possibly impair meristematic tissue sensitivity to the growth signals. Tissue sensitivity to cytokinins is important for the regulation of dormancy, as

an increase in both cytokinin content and sensitivity appear to be the principal factors leading to the loss of dormancy (Turnbull and Hanke, 1985; Suttle, 2004b). Cessation of meristematic activity in potato stolon apex during tuberization has been reported (Burton, 1989) and this can be orchestrated by ethylene inhibition of the action of cytokinins (Dimalla and van Staden, 1977). It is therefore possible that exogenous ethylene elicits a similar effect in sweetpotato during the ecodormancy phase. More recently, Bromley et al. (2014) isolated from potato shoot apices a cytokinin-sequestering protein (cytokinin riboside phosphorylase), which down-regulated tissue sensitivity to cytokinins. A similar cytokinin-sequestering substance is possibly induced in sweetpotato in the presence of exogenous ethylene which forms a complex with ZR and inhibits its action until ethylene is removed. The ensuing metabolism may result in compensatory growth due to activation of meristematic tissue sensitivity to the highly accumulated ZR. This may explain the proliferous sprout growth when the ethylene source is removed.

Changes in the level of the plant growth regulator abscisic acid (ABA) in sweetpotato in relation to the dormancy mechanism has not been reported previously. ABA is classified alongside ethylene as a growth retardant and is therefore implicated to have a role in the sprout growth inhibition complex. Many reports point to dormancy break as being associated with reduced ABA concentration although the threshold level is uncertain. ABA interaction with ethylene in regulating endodomancy is, to date, not known (Suttle, 1998). In the study herein, the dry mass concentration of ABA in both tissues were initially high (487 μg kg^{-1} and 345.2 μg kg^{-1} in the skin and flesh, respectively). Over time, the concentrations sharply declined in both continuous ethylene treated and control roots (Figure 2). Similar declines in the content of ABA

during storage has been reported in potato cv. Marfona (Ordaz-Ortiz et al., 2015; Foukaraki et al., 2016a) and in onion cvs. Red Baron, Sherpa and Wellington (Chope et al., 2006). It was also reported that ABA concentration in onion (Chope et al., 2006) and potato (Coleman and King, 1984) were higher at the low temperatures of 4 ^{o}C and 2 ^{o}C, respectively, but the levels dropped when both crops were stored at 20 ^{o}C, indicating the importance of temperature in also regulating ABA levels. The decline in ABA levels observed in sweetpotato in the present study may therefore be partly as a result of the higher storage temperature of 25 ^{o}C. Exogenous ethylene, compared to air storage, caused greater decline in the levels of ABA in sweetpotato. Immediately after dormancy break, ABA content in the skin tissues of exogenous ethylene-treated roots stabilized; and this was also laterly observed in the flesh tissues. According to Suttle (2004a), while both ethylene and ABA are needed to initiate dormancy in potato, only ABA is required to sustain the dormant state. Thus the inhibition of ABA in the meristematic skin tissues of sweetptotato by exogenous ethylene could explain the early termination of dormancy while sustained exposure to ethylene explains the failure of sprouts to grow as discussed previously. On the other hand, a related study by Foukaraki et al. (2016a) of endogenous ABA response to exogenous ethylene in potato cv. 'Marfona' showed a rise in the concentration of ABA which, the authors suggested, may account for the inhibition of sprout growth.

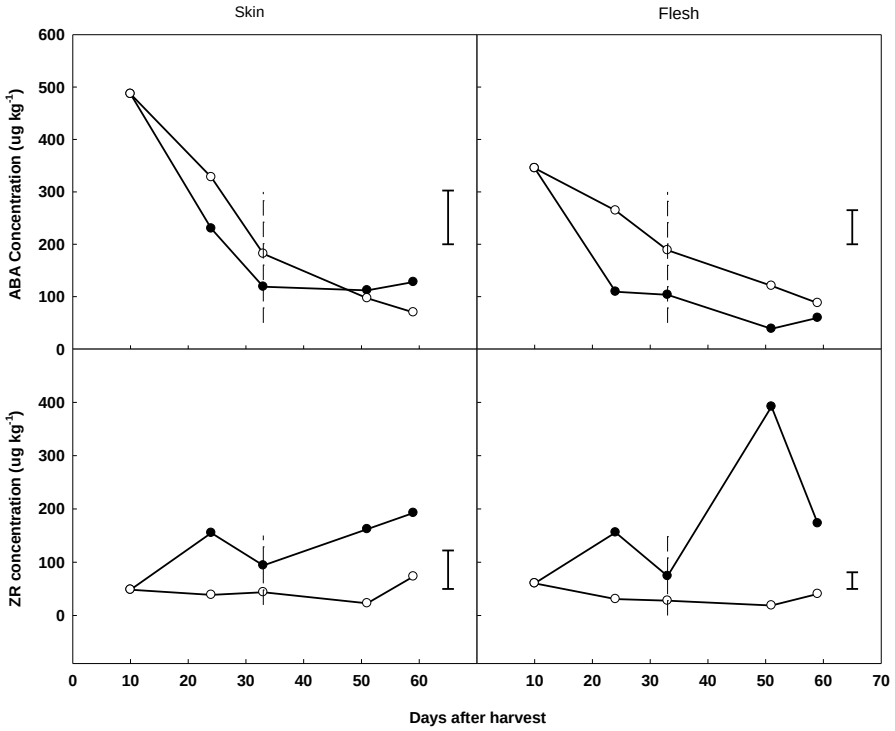

Figure 2. Effect of ethylene (10 μL L^{-1}) on the dry mass concentration (μg kg^{-1}) of ABA and ZR in the proximal section of North Carolina-derived sweetpotato cv. Covington (Experiment 2a). Roots were treated 10 days after harvest followed by storage at 25 ^{o}C. (●) Continuous ethylene storage; (○) continuous air storage. Dormancy break is shown as vertical broken lines. The l.s.d. $_{(0.05)}$ bars are for time-treatment interaction effects.

3.3 Weight loss

While reduced sprout growth may be advantageous for sweetpotato stored, the benefits were reduced by the finding that roots flushed with continuous ethylene lost weight at significantly faster rates (ca. 1.4-fold) than those stored in air ($P < 0.01$). Mean weight loss during the first 4 weeks in air storage were 9.8 %, 3.6 % and 7.8 % in Experiments 1, 2a and 2b, respectively, while the corresponding losses due to ethylene supplementation were 13.1 %, 5.9 % and 10.1 %, respectively (Figure 3). The accelerated weight loss in ethylene were largely linked to increased moisture loss. Moisture loss could also be accentuated by elevated temperature, increased respiration

and low ABA concentration. The older roots in Experiments 1 and 2b lost weight at much faster rates than the freshly harvested roots used in Experiment 2a. There was a significant benefit in weight loss reduction when the roots were supplemented with ethylene after dormancy break compared to continuous treatment from the beginning of storage.

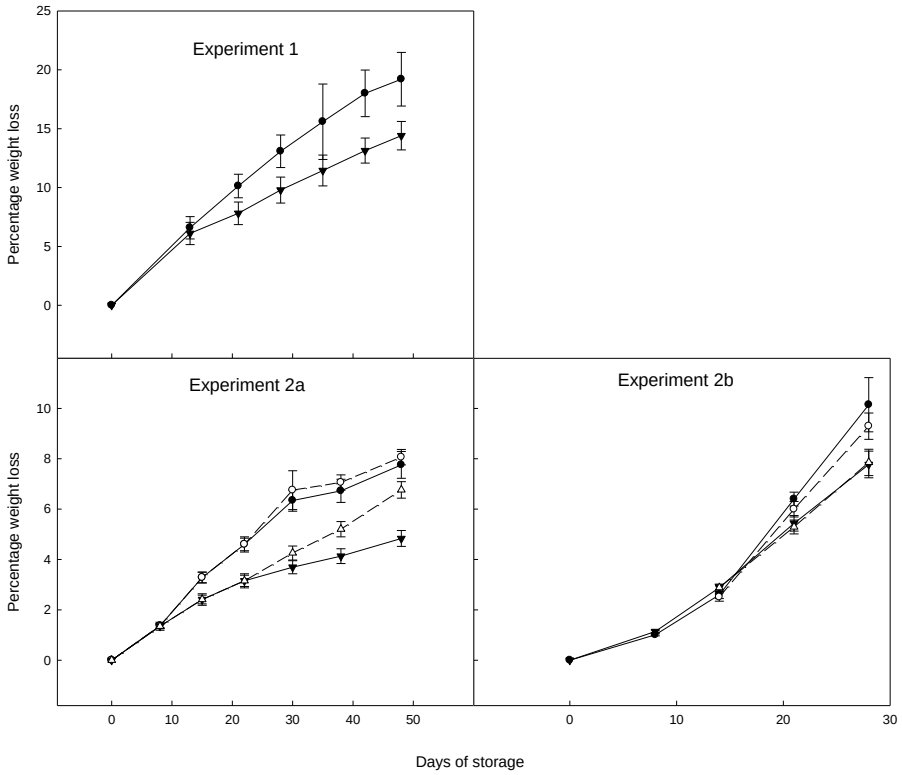

Figure 3. Effects of ethylene (10 μL L^{-1}) on weight loss of North Carolina-derived sweetpotato cv. Covington during storage. (●) continuous ethylene; (○) ethylene followed by air; (▼) continuous air; (Δ) air followed by ethylene. Roots for Experiment 2a were treated 10 days after harvest followed by storage at 25 ^{o}C. Roots for Experiment 2b were initially held in cold storage at 14 ^{o}C (150 days) followed by the treatments and storage at 25 ^{o}C. Error bars are shown.

3.4 Root decay

No incidence of decay was recorded in the freshly harvested roots used in Experiment 2a within the evaluation period. In contrast, the incidence of disease (black rot and soft rot) in the older roots from Experiments 1 and 2b was differentially influenced by the treatments (Supplementary Figure 1). Exogenous ethylene supplementation tended to escalate decay; the roots of Experiment 2b incurring ca. 2-fold greater incidence of disease in ethylene-treated than control roots. This demonstrates that ethylene supplementation may have to be integrated with other methods for curbing disease. Ethylene effects on postharvest decay vary depending on the crop-pathogen system and may be the result of direct interaction with the pathogen or by modification of crop metabolism. Ethylene reportedly enhances resistance of sweetpotato against black rot (Stahmann et al., 1966) but in other studies, it promoted root decay (Arancibia et al., 2013). It was noted in the current study that ethylene-induced decay predominantly occurred at the proximal ends (tip-rot) where root splitting was also observed, although in some roots, the decay affected other parts as well. This is consistent with the report by Arancibia et al. (2013) who found that pre-harvest foliar application of sweetpotato cvs. Beauregard and B-14 with the ethylene analogue ethephon increased the incidence of root tip rot. The increased disease incidence in the proximal region may be related with the tissue integrity and the proximal root splitting.

3.5 Effect of exogenous ethylene on non-structural carbohydrates

In contrast to potato in which exogenous ethylene reportedly can cause accumulation of the monosaccharides fructose and glucose (Prange et al., 2005; Foukaraki et al., 2014; Foukaraki et al., 2016b), evidence from the present work supports the finding by

Cheema et al. (2013) that ethylene tends to elicit the opposite effect in sweetpotato. Thus exogenous ethylene inhibited increase of fructose and glucose in both Experiment 2a and Experiment 2b (Figure 4 and Supplementary Figure 2). Indeed in Experiment 2b, glucose was significantly suppressed in the flesh tissues by continuous ethylene. In a similar study, Chegeh and Picha (1993) found no change in the contents of fructose and glucose in cured and non-cured cvs. Beauregard and Jewel when stored for 15 days at 21 ^{o}C in a range of ethylene concentrations from 0 μL L^{-1} to 1000 μL L^{-1}.

Dormancy break in Experiment 2b was associated with a transitory increase in the monosaccharides within the control flesh tissues to peak values. This may be associated with the energy requirement for dormancy release and sprout growth. The peaking of fructose was less pronounced compared to that of glucose.

While accumulation of monosaccharides was inhibited in ethylene supplemented roots, the treatment tended to boost sucrose concentration by 17.3 % and 3.0 % in Experiment 2a and 2b, respectively, compared to storage in air. This result is consistent with Chegeh and Picha (1993) and Cheema et al. (2013) who found an increase in the sucrose level in ethylene treated roots. Sucrose mobilisation for unloading into growing buds has been explained as a mechanism to meet the energy demand for bud outgrowth in potato (Sonnewald and Sonnewald, 2014).

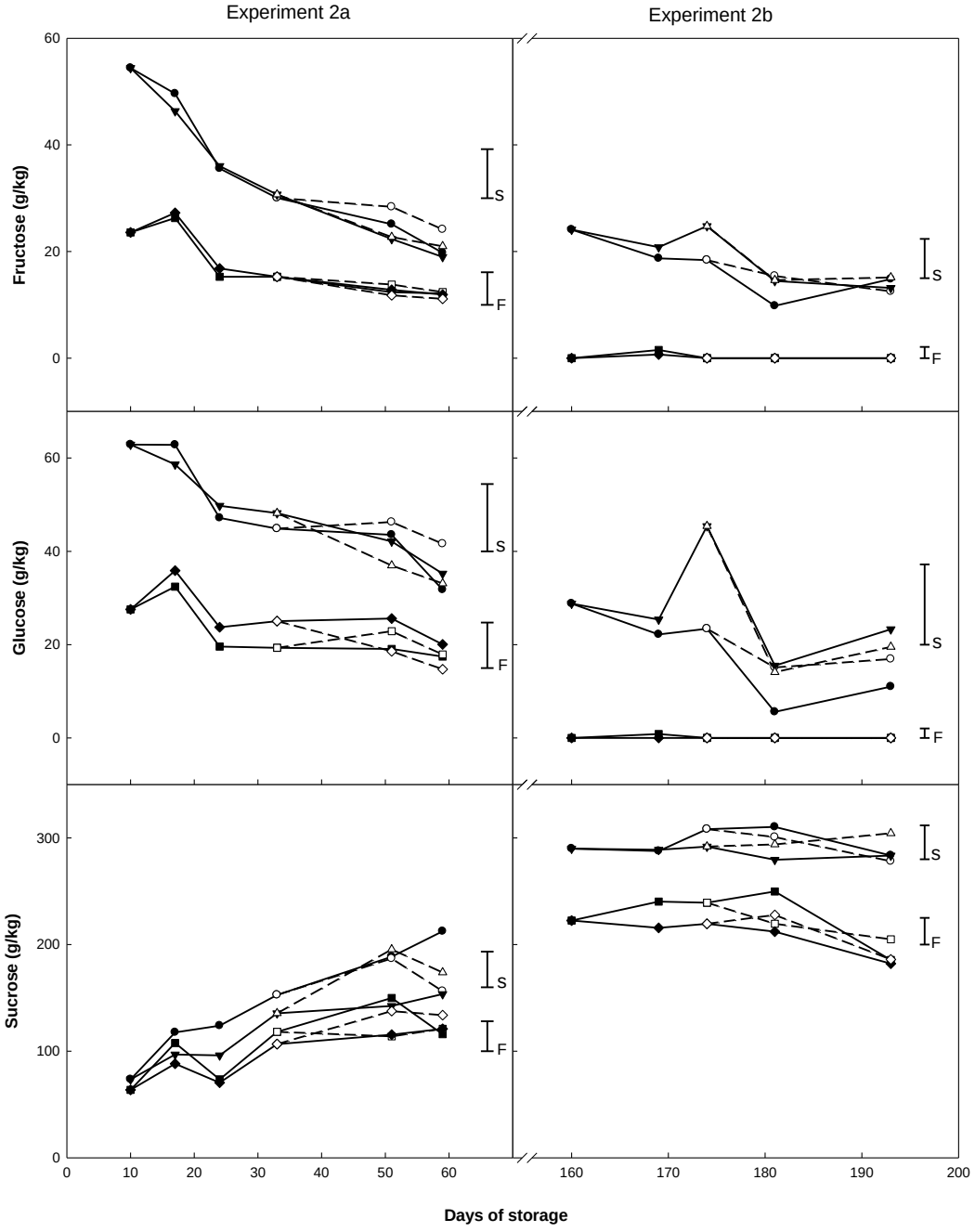

Figure 4. Effects of ethylene (10 µL L^{-1}) on dry mass concentration of sugars in the proximal section of North Carolina-derived sweetpotato cv. Covington during storage. (●) continuous ethylene (flesh); (○) ethylene followed by air (flesh); (▼) continuous air (flesh); (Δ) air followed by ethylene (flesh); (■) continuous ethylene (skin); (□) ethylene followed by air (skin); (♦) continuous air (skin); (◊) air followed by ethylene (skin). Roots for Experiment 2a were treated 10 days after harvest followed by storage at 25 ^{o}C. Roots for Experiment 2b were initially held in cold storage at 14 ^{o}C (150 days) followed by the treatments and storage at 25 ^{o}C. L.s.d. $_{(0.05)}$ bars shown are for treatment-time interaction effects for skin (S) and flesh (F) tissues.

3.6 Effect of exogenous ethylene on phenolic compounds

The content and spatial distribution of the total phenolics in sweetpotato have been determined by many authors (Walter and McCollum, 1979; Truong et al., 2004; Teow et al., 2007; Truong et al., 2007 and Jung et al., 2011). There is, however, scarcely any published work on the spatio-temporal changes of individual phenolic compounds during storage. Major phenolic compounds found in the North Carolina-derived Covington consignments were chlorogenic acid and its isomers (isochlorogenic acids A, B and C), caffeic acid and coumaric acid (Figures 5, 6 and Supplementary Figure 3). These compounds have been reported to be present in considerable quantities in many genotypes of sweetpotato (Truong et al., 2004; Nandutu et al., 2007; Jung et al., 2011; Truong et al., 2007; Teow et al., 2007). Chlorogenic acid content was the highest in flesh tissues (baseline concentrations 169 mg kg^{-1} and 74.8 mg kg^{-1} in Experiments 2a and 2b, respectively). Chlorogenic acid also dominated in the skin of roots in Experiment 2a (baseline concentration 896 mg kg^{-1}) but isochlorogenic A (baseline concentration 720 mg kg^{-1}) was the dominant phenolic compound in the skin of roots used in Experiment 2b, followed by chlorogenic acid. There was ca. 8-fold greater concentration of phenolics in the skin than the flesh tissues; and 1.4-fold greater level in the proximal compared to distal root sections. Jung et al. (2011) also reported that the level of phenolic compounds in sweetpotato was highest in the proximal region. Chlorogenic acid is the most important cinnamic acid-derivative and its role in plants as phytoanticipin is well documented (Harbone, 1994). The greater abundance of phenolics in the skin tissues is therefore likely associated with their role in contributing to natural disease resistance (Schwalb and Feucht, 1999). Caffeic acid was almost absent from all

flesh tissues of the control roots throughout storage (Figure 6). The concentration of all the phenolic compounds increased during the initial storage in the fresh roots (Experiment 2a) but declined after long-term storage (Experiment 2b). Coumaric acid was only found in the fresh roots (Experiment 2a) and not in the 5 months stored roots (Experiment 2b).

Dormancy break was marked with accentuated increases in the contents of all phenolic compounds in both ethylene and air treatments. This change suggests the possible association of phenolics in sweetpotato sprout development.

Ethylene supplementation reportedly boosted the total phenolics concentration in sweetpotato (Buescher et al., 1975; Buescher, 1979 and Walter and Purcell, 1980). It was noted in the current study, however, that synthesis of the individual phenolic compounds was differentially affected by ethylene depending on the compound-tissue-section matrix. Ethylene supplementation significantly increased the concentration of isochlorogenic acid C in both experiments by ca. 1.7-fold in the skin tissues while in the flesh tissues, the concentrations rose by ca. 10-fold. Isochlorogenic acid A concentration was also significantly increased in both tissues of Experiment 2b but only in the flesh tissues of Experiment 2a. Chlorogenic acid was not significantly affected by exogenous ethylene except in the proximal flesh tissues of the previously stored roots used in Experiment 2b. Likewise, while there was no significant effect of exogenous ethylene on isochlorogenic acid B in Experiment 2a, a delayed increase in the distal skin tissues was observed in Experiment 2b. While ethylene supplementation tended to boost the concentrations of chlorogenic acid derivatives, it suppressed the contents of caffeic (Figure 6) and coumaric acids (Supplementary Figure 4). This was evident from the

chromatographic peaks (not shown), in which there was reciprocity between chlorogenic acid and caffeic acid accumulation.

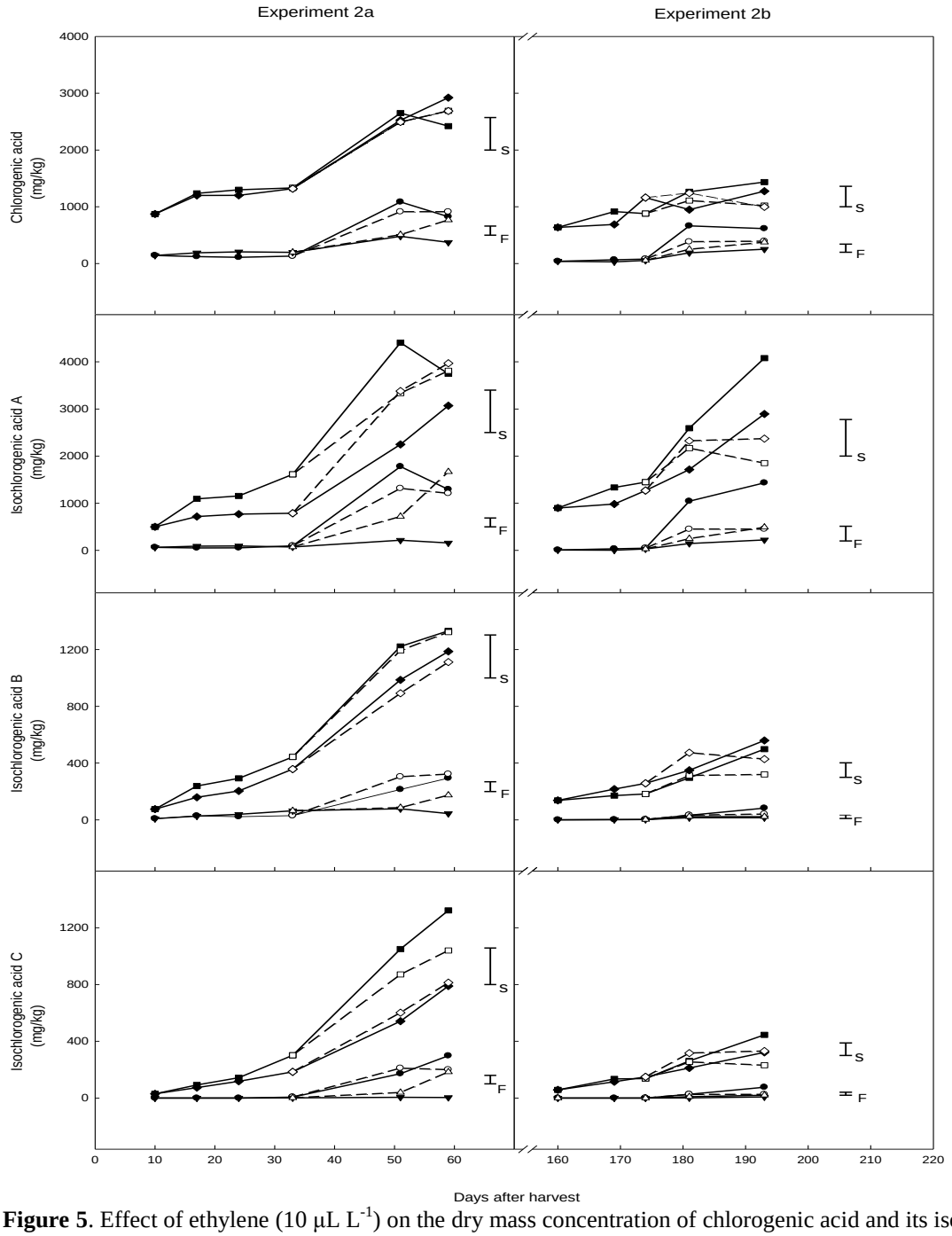

Figure 5. Effect of ethylene (10 μL L^{-1}) on the dry mass concentration of chlorogenic acid and its isomers in the proximal section of North Carolina-derived sweetpotato cv. Covington during storage. (●) continuous ethylene (flesh); (○) ethylene followed by air (flesh); (▼) continuous air (flesh); (Δ) air followed by ethylene (flesh); (■) continuous ethylene (skin); (□) ethylene followed by air (skin); (♦) continuous air (skin); (◊) air followed by ethylene (skin). Roots for Experiment 2a were treated 10 days

after harvest followed by storage at 25 ^{o}C. Roots for Experiment 2b were initially held in cold storage at 14 ^{o}C (150 days) followed by the treatments and storage at 25 ^{o}C. L.s.d. $_{(0.05)}$ bars shown are for treatment-time interaction effects for skin (S) and flesh (F) tissues.

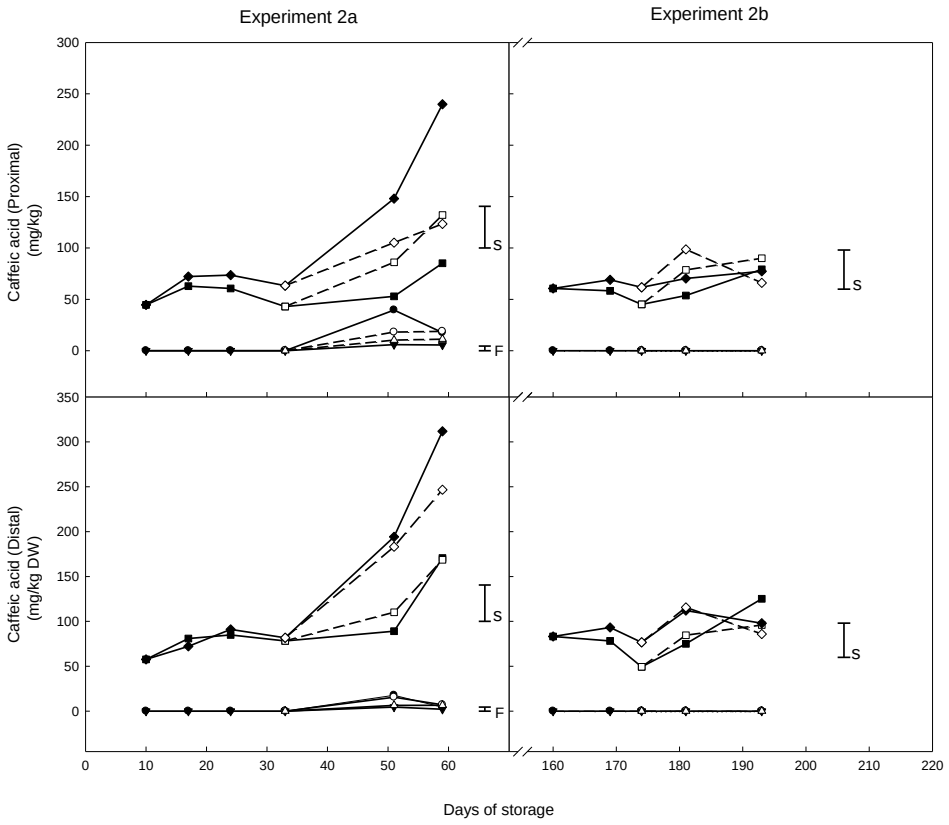

Figure 6. Effect of ethylene (10 µL L^{-1}) on the dry mass concentration of caffeic acid in North Carolina-derived sweetpotato cv. Covington. (●) continuous ethylene (flesh); (○) ethylene followed by air (flesh); (▼) continuous air (flesh); (Δ) air followed by ethylene (flesh); (■) continuous ethylene (skin); (□) ethylene followed by air (skin); (♦) continuous air (skin); (◊) air followed by ethylene (skin). Roots for Experiment 2a were treated 10 days after harvest followed by storage at 25 ^{o}C. Roots for Experiment 2b were initially held in cold storage (150 days) at 14 ^{o}C followed by the treatments and storage at 25 ^{o}C. L.s.d. $_{(0.05)}$ bars shown are for treatment-time interaction effects for skin (S) and flesh (F) tissues.

4 Conclusion

In conclusion, the swap treatments used in this study are a novel approach that enabled the dynamic effects of ethylene on sweetpotato to be appropriately tracked. Ethylene

supplementation just after early signs of dormancy break appears to be a practically more suitable approach as it achieved an equivalent effect in suppressing sprout growth as continuous ethylene application from the beginning of storage. Post-dormant ethylene supplementation had additional benefits in limiting ethylene-induced rot symptoms, weight loss and potential cost. Ethylene-stimulated rise in the level of the growth hormone zeatin riboside without concomitant sprout growth suggests the presence of a cytokinin sequestering substance concurrently promoted by ethylene.

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